Mycopath (2007) **5**(2)**:** 107-110

Comparison of Phytochemical and chemical control of *Fusarium oxysporium* f. sp. *ciceri*

Irum Mukhtar

Institute of Mycology and Plant Pathology, University of the Punjab Lahore, Pakistan
E.mail: erumm21@yahoo.com

Abstract

The antifungal effect of aqueous extracts of four plant species viz; *Azadaracta indica* A. Juss., *Datura metel* L. var. *quinquecuspida* Torr., *Ocimum sanctum* L. and *Parthenium hysterophorus* L. was determined *in vitro* study. It was found that all the plant extracts at 40% concentration were effective in reducing the mycelial growth *of F. oxysporum* f. *sp. ciceri*. Among these plants extracts, *A. indica* and *D. metel* inhibited fungal growth by 80%. Even at 10% concentration, both plants extracts had inhibitory effect, while *Ocimum sanctum* extracts showed low inhibition (60%) as compare to other plant extracts. Chemical treatment with Benomyl (50WP) and Carbendazim (50WP) was proved to be the most effective against *F. oxysporium* f. sp. *ciceri*. Results indicated, plant extracts had equal potential as fungicides for the reduction of pathogen growth.

Keywords: Antifungal effect, Chickpea wilt, Fungicides, *Fusarium oxysporum*

Introduction

Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop, after beans (*Phaseolus vulgaris* L.) and peas (*Pisum sativum* L.) (Vishwadhar and Gurha, 1998). Fusarium wilt [*Fusarium oxysporum* f. sp. *ciceri* (Padwick) Matuo and K. Sato)] is one of the major yield limiting factors of chickpea (Jalali and Chand, 1992; Dubey *et al.*, 2007). In Pakistan, during 2005-6, chickpea was grown on an area of 1066 thousands ha with a production of 536 thousand tons. Punjab and Sindh with an area of 928 thousand and 52 thousand ha respectively are the leaders in chickpea production (Anonymous, 2007). In Pakistan, Fusarium wilt causing 10-50% yield losses in dry areas during the last several years, while in irrigated belts of Punjab, farmers have shifted to other crops due to this disease (Ikramul and Farhat, 1992). In order to prevent the plant diseases and to protect the crop plants against pathogens, chemical control methods are in practice. In view of the high cost of chemical pesticides and their hazardous consequence use of biodegradable material like fresh plant extracts from parts gained importance during last three decades from plant disease control (Grainge and Ahamed, 1988; Jespers and Ward, 1993). The use of chemicals has helped increase of yields obtained but one of the major problems with the constant use of chemicals is that resistance can be induced in target organisms and contaminate the environment with very toxic substances (Okigbo, 2004; Carvalho, 2004). A possible alternative to solve such problem is the use of plants able to produce antifungal substances (Miranda, 2003). Fungicides of plant origin are environmentally safe and nonphytoxic. The extract of these plant materials can be easily prepared by farmers (Okigbo and Nameka, 2005). On the basis of efficacy of plant extracts, present study was conducted to find out the effective plant product for the management of *Fusarum oxysporum* f. sp *ciceri* and to determine comparison of antifungal compound to fungicides.

Materials and Methods

Source and maintenance of Microorganism

A virulent, wilt causing isolate of *Fusarium oxypoium* f. sp.*ciceris* (2012) was obtained from Agricultur University of Faisalabad, Pakistan and maintained on Malt Extract Agar medium(MEA) at 27^{o}C.

Preparation of plant extract

Fresh leaves of four plants viz. *Azadaracta indica*, *Datura metel*, *Ocimum sanctum* and *Parthenium hysterophorus* were collected from Quid-e-Azam campus, University of the Punjab, Pakistan. Collected plant materials were surface sterilized with 0.1% sodium hypochlorite and repeatedly washed in sterile water and cut into small pieces. A 50% w/v stock solution of *A. indica*, *D. metel*, *O. sanctum* and *P. hysterophorus* was prepared by soaking the crushed plant materials in sterilized water for 24 h at room temperature, passing through muslin

cloth and finally through Whatman filter paper No.1. The lower concentrations of 10%, 20%, 30% and 40% w/v were prepared by adding appropriate quantity of sterile water into the stock solution. The diluted plant extracts were heated to 40-50^{o} C for 10 min. to avoid contamination (Jagannathan and Narasimhan, 1987). The extract was stored at 4°C to avoid contamination and prospective chemical alterations.

Preparation of Fungicides

In vitro, three fungicides were used against *Fusarium oxysporum* f. sp *ciceri* viz., Benomyl 50%WP, Captan 2 %(2gm/kg seed) and Carbendazim. 50%WPof each fungicide was used for 0.1%, 0.2% and 0.3% dilutions of each fungicide.

Assay of plant extract

The efficacy of *A. indica*, *D. metel*, *P. hysterophorus* and *O. sanctum* extracts was tested *in vitro*, against the pathogen following the poisoned food technique (Mishear and Tiwari, 1992). Aqueous extract bioassays were carried out in solid medium. Two percent of Malt extract agar (MEA) medium was prepared in 250 mL conical flasks. To avoid bacterial contamination, antibacterial chloromycetin capsules were used. For 10-40% concentrations of each test plant species, appropriate quantity of 50%(w/v) stock solution was mixed in molten sterile MEA medium at 40^{o} C and pored into sterilized glass petri plates @ 20ml per plate. Sterile distil water was added in control plates. After the solidification of the medium, 1 cm diameter plug from 7-day-old colony of *F. oxysporium* was inoculated aseptically in the center of each petriplate and incubated at 27^{o} C. The colony diameter of *F. oxysporium* was measured after 7 days of incubation. Three replicates in a completely randomized design were used within each treatment.

Assay of fungicides

0.1%, 0.2% and 0.3% dilution of each fungicide viz., Benomyl, Captan and Carbendazim was prepared by adding appropriate amount of fungicides in molten agar at 40^{o} C and pored into sterilized glass petri plates @ 20ml per plate. Each plate was inoculated with a 1 cm diameter plug of agar that had been colonized by the *F. oxysporium*, and plates were sealed with parafilm to prevent dehydration. Inoculated plates were incubated for 7 days at 27^{o} C. Three replicates in a completely randomized design were used within each treatment. The percent inhibition of mycelia growth over control was calculated using the formula (Vincent, 1947):

$$\% \text{ of inhibition} = \frac{\text{Diameter of control colony} - \text{Diameter of treated colony}}{\text{Diameter of control colony}} \times 100$$

Results and Discussion

All the plant products tested significantly reduced the fungal growth as against 87 mm radial growth in control treatment (Fig. 1). Among these, 40% of *A. indica* and *D. metel*, cause 80% growth inhibition of *Fusarium oxysporium* fr. *cieris*. 40 % plant extracts of *P. hysterophorus* (75%) and *O. sanctum* (72%) were moderately effective to control the mycelial growth of *F. oxysporium* fr. *cieri*. In lower concentrations, 10% of *A. indica* and *D. metel* showed effectiveness in minimizing the colony growth of fungi up to 69% to 68% in that order followed by *P. hysterophorus* (66%).

Statistically analyzed result clearly indicated the higher fungitoxicity of *A. indica* and *D. metel* extract to control mycelial growth of the *F. oxysporium* fr.*cieri*. It has also been studied earlier that the plant extracts, viz *Calotropis procera*, *Eucalyptus globulens*, *Jatropha multifida Azadirachta indica*, *Allium sativum* were significantly pronounced in reducing wilt incidence in *Cicer arietinum* L. (Chand and Singh, 2005). Results also indicated that the 30% all plant extract were equally inhibited the fungal growth *in vitro* (Fig.1). Mycelial growth of various *Fusarium* species were inhibited by the plant extracts of *Adhatoda vasica*, *Azadirachta indica*, *Cinnamomum camphora* and *Ocimum sanctum* (Prasad and Ojha, 1986); *Agave americana*, *Cassia nodosa* (Reddy and Reddy, 1987); *Azadirachta indica* (Eswaramoorthy *et al*., 1989); *Azadirachta indica*, *Atropha belladona*, *Calotropis procera*, *Eucalyptus amygdalina*, *Ailanthus excelsa* and *Lantana camera* (Bansal and Rajesh, 2000). Nwachukwu and Umechuruba (2001) also reported, *Azadirachta indica* extract was the most effective on major seed-borne fungi. Jain (2003) found *Parthenium hysterophorus* to completely inhibit the growth of *Fusarum solani*. Analysis of Variance shows that effect of test chemicals, different empolyed doses significantly reduced the fungal growth.At higher concentration, Benomyl and carbendazim were the most effetive fungicides among the tree fungicides used. Captan was least effective in higher concentration (Fig. 2). Similarly, Cother (1977) obtained effective control of gram wilt by seed treatment with Benomyl, Captan and Thiram. Gupta *et al*. (1997) screened 6 fungicides against *F. oxysporium* f. sp. *ciceri* and reported Carbendazim @100 mg/ml as most

effective in inhibiting the growth of fungus *in vitro*. However, at low concentration, test fungicides showed equally effective behavior. Christian *et al.* (2007) also reported significant effect of five fungicides, Benomyl (1 mg/l), dodine (50 mg/l), Manzate (100 mg/l), Cupric sulphate (200 mg/l) and thiabendazole (4 mg/l) under *in vitro* conditions on growth of 28 isolates of pathogenic fungi.The highest inhibition of fungi was obtained with Cabindazim> Benomyl and Captan (Fig.2). Chemical Seed treatment with Thiram (0.15%) + Carbendazim (0.1%) were found most effective against *F. oxysporium* f. sp. *ciceri* (Nikum *et al.,* 2007). Carbendazim 0.2% was effective among three treatments (Fig. 2). De *et al.,* (1996) also found that coating of chickpea seeds with Carbendazim (0.2%) was more effective in reducing wilt and increasing seed yield by 25.9 to 42.6 percent.

As compare to plant extracts, 40% of *A. indica*(80%) is equally effective as Benomyl and carbendazim. lower concentration (10%) of *A. indica*, *D. metel* and *P. hysterophorus* are more effective as compare to lower concentration of Captan, Benomyl and carbendazim. Joseph (2003) reported that half the recommended dosage of Benomyl (0.3 g per L) combined with 1% (w/v) garlic extract was as effective as the full dosage of the fungicide, which gave complete control of *Colletotrichum capsici.* The present study signifies the importance of aqueous extracts plants as potential agent to be manipulated for biological control of *F. oxysporum* f. sp *ciceri* as they are equally effective as the fungicides. Biological method of disease control should be preferred if specific formulation is effective against the pathogen.

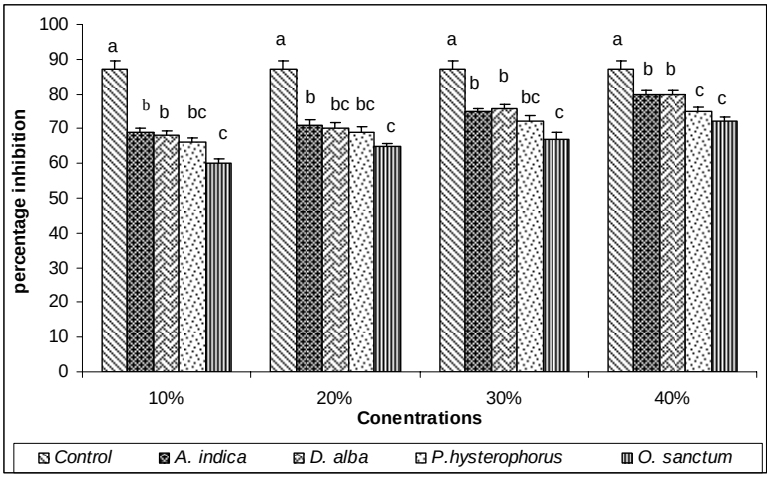

Fig 1. Percentage inhibition of *Fusarium oxysporium* by different Plant extracts *in vitro*. Verticals bars show standerd error of means of threereplicales.values with different letters in a column shows siginficant difference as determin by Duncan' s Mulptipul Range Test at P≤0.05.

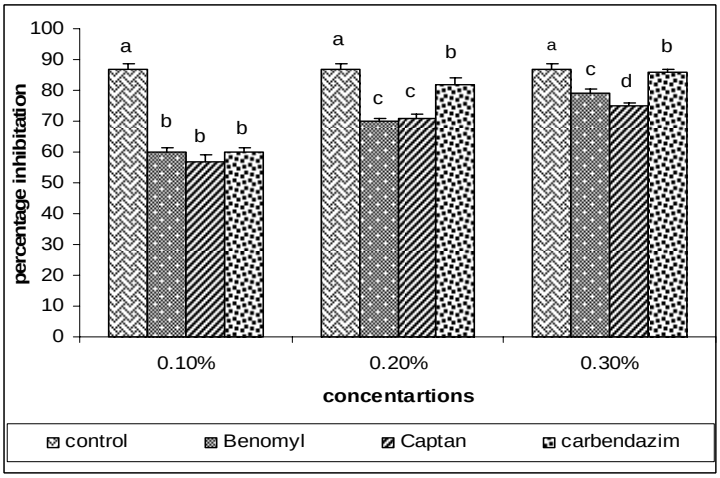

Fig 2. Percentage inhibition of *Fusarium oxysporium* by different chemicals *in vitro.* Verticals bars show standerd error of means of threereplicales.values with different letters in a column shows siginficant difference as determin by Duncan s Mulptipul range Test at P≤0.05.

References

Anonymous, 2007. Agricultural Statistics of Pakistan. Ministry of Food, Agric. and Livestock, Govt. of Pakistan, Islamabad.

Bansal KR, Rajesh KG, 2000. Evaluation of plant extracts against Fusarium oxysporum, wilt pathogen of fenugreek, *Indian. J. Phytopathol*. **53 (1):**107-108.

Carvalho GA, 2004. Filtered effect in vitro and in alive of of *Gloeosporioides rizobacteria* on *Colletotrichum penz*. of the cofee tree. P.55 Dissertação (Mestrado in Agronomy) - Federal University of YOU cultivate, You cultivate.

Chand H, Singh S, 2005.Control of chickpea wilt (*Fusarium oxysporum* f sp *ciceri*) using bioagents and plant extracts. *Indian J. Agric. Sci.* **75 (2)**: 115-116.

Christian L, Morel N, Luiz R, 2007. In vitro susceptibility to fungicides by invertebrate-pathogenic and saprobic fungi. *Mycopathologia*. **164(9)**: 39-47.

Cother EJ, 1977. Identification and control of root rot fungi in gram. *Plant Dis. Reptr*., **61**: 376

Dubey SC, Suresh M, Singh B, 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt**.** *Biol. Control.* **40 (1):** 118-127.

Eswaramoorthy S, Muthusamy S, Mariappan V, 1989. Neem, *News letter*, **6 (1)**: 4-5

Gupta SK, Upadhyay JP, Ojha KH, 1997. Effect of fungicidal seedtreatment on the incidence of chickpea wilt complex. *Ann. Pl. Prot. Sci*. **5**: 184-187.

Grainge M, Ahmed S, 1988. Hand Book of Plant pest control properties (John Wiley and Sons, NewYork) 470

Ikramul H, Farhat FJ, 1992. Screening of chickpea lines in the wilt sick plot and effect of environmental temperature on wilt incidence. Proceedings of COMSTECH-NIAB International Workshop on Agroclimatology, pests and diseases and their control. November 21-26, 1992, Faisalabad, Pakistan.

Jain C, 2005. Search of natural pesticides of local weed plants**.** *Asian J. Microbiol. Biotechno. Environ. Sci.* **7 (2)**: 331-332.

Jalali BL, Chand H, 1992. Chickpeawilt. pages 429-444 in: Plant Diseases of International Importance. Vol. I. Diseases of Cereals and Pulses. U. S. Singh, A. N. Mukhopadhayay, J. Kumar, and H. S. Chaube, eds.Prentice Hall, Englewood Cliffs, NJ.

Jespers ABK, Ward MA, 1993. Natural Products in Plant Protection, *Netherland, J. Plant Pathol.* **99 (3)**: 109 –117.

Joseph O, 2003. Control of brown blotch of bambara groundnut with garlic extract and Benomyl. *Phytoparasitica*. **31(2):** 207-209.

Miranda EFO, 2003. Morphologic, molecular characterization, pathogenic biochemist and of isolated of *Colletotrichum spp*. associates to the cofee tree in *Minas Gerais* and comparison with *Colletotrichum Kahawae*. P.147. Thesis (Doutorado in Agronomy) - Federal University of You cultivate, You cultivate.

Nikam PS, Jagtap GP, Sontakke PL, 2007. Management of chickpea wilt caused by *Fusarium oxysporium* f. sp. *ciceri. African J. Agric. Res.* **2 (12)**: 692-697.

Nwachukwu EO, Umechuruba CI, 2001. Antifungal activities of some leaf extracts on seed-borne fungi of African yam bean seeds, seed germination and seedling emergence. *J .App. Sci. Environ. Manage*. **5(1)**: 29-32.

Okigbo RN, 2004. A review of biological control methods for post harvest yams *Dioscorea* spp.) in storage in South Eastern Nigeria. *KMITL Sci J.* **4(1)**: 207 - 215.

Okigbo RN, Nmeka IA, 2005 . Control of yam tuber with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale*. *Afr. J. Biotechnol***. 4(8)**: 804 – 807.

Prasad AK, Ojha NL 1986. Antifungal evaluatiion of leaf extracts for the control at some cucurbitaceous fruit rot diseases. *Indian Phytopathol.* **39** :153

Reddy VK, Reddy SM, 1987. Screening of indigenous plants for their antifungal principle. *Pesticides*, **21**:17-18

Vincent JM, 1947. Distoration of fungal hyphae in the presence of certain inhibitors. *Nature*, **159**: 850

VishwaDhar, Gurha SN, 1998. Integrated Management of Chickipea Diseases. Integrated Pest and disease management. Rajeev K, Upadhyay KG, Mukerji BP, Chamola and Dubey OP (eds.) APH Publishing Co., New Delhi. (India). p. 249.